Laboratory Report

# Studies of Sulfite Reactivity in Metabisulfite Containing Propofol Emulsions

December 1, 2001

Max T. Baker, PhD

1. Sulfite radical formation in sulfite containing propofol emulsion

2. Genesis of propofol emulsion yellowing and oxidative processes in a simulated 12 hour intravenous drip

## 1. Sulfite radical formation in sulfite containing propofol emulsion

It has previously been demonstrated that propofol is oxidized to a propofol dimer in commercial metabisulfite containing propofol emulsions after the vial is opened and the emulsion exposed to air[1]. This does not occur in commercial EDTA containing propofol emulsion indicating that sulfite functions to catalyze this propofol oxidation.

It has also been demonstrated that there are detectable concentrations of malondialdehyde (MDA) in commercial sulfite containing propofol emulsions when the vials are immediately analyzed for MDA after opening (Baker manuscript submitted, appendix). MDA, a marker of lipid peroxidation, is not detectable in EDTA propofol emulsion similarly analyzed. These finding point to the fact that metabisulfite ($Na_2S_2O_3$), which dissociates to sulfite ($SO_3^{-2}$) in solution, is in some manner catalyzing both of these oxidative processes. Sulfite has been reported to form free radicals in the presence of oxygen, and it is hypothesized that initial sulfite oxidation to free radicals is the mechanism by which a strong oxidative environment is created in the sulfite containing propofol emulsion.

To this end, electron spin resonance (ESR) spectrometry was performed on sulfite containing propofol emulsion and EDTA containing propofol emulsion. This was done by withdrawing 500 µl emulsion from fresh unopened vials (1% propofol emulsion, Gensia Sicor Pharmaceuticals Inc, lot OOE318, exp 05/02; Diprivan, AstraZeneca Pharmaceuticals, lot 4017F, exp 06/03), mixing the emulsion with an electron spin trapping agent (100 mM DMPO), and immediately scanning for ESR signals.

As shown in figure 1, a strong free radical signal is detected in sulfite containing propofol emulsion. In contrast, there is no radical signal in EDTA propofol emulsion identically analyzed (Figure 2). Additionally, when the emulsions are bubbled with 95% oxygen prior to analysis, no signal is detected in EDTA propofol emulsion, whereas a strong signal is otherwise observed in sulfite containing propofol emulsion.

Hyperfine splitting values for the radical signal in the sulfite containing propofol using DMPO as trap were: $a^H$ = 15.79 to 16.11 G, and $a^N$ = 14.60 to 14.71 G. This radical ESR spectrum is consistent with literature ESR spectra of the sulfite anion radical $(SO_3^-)$ [2]. The occurrence of this radical indicates that in the sulfite containing propofol emulsion, sulfite is readily oxidized to the sulfite free radical when air is introduced. Furthermore, it shows that radical is formed in spite of the fact that propofol, which functions as an antioxidant[3] is present.

The scheme below depicts the formation of the sulfite radical upon after reaction between sulfite anion and oxygen. It is this proposed reaction of sulfite with oxygen in the sulfite propofol emulsion that is the initial step in the cascade of reactions leading to lipid and propofol oxidation.

$$SO_3^{-2} + O_2 \rightarrow SO_3^- + e^-$$

## 2. Genesis of propofol emulsion yellowing and oxidative processes in a simulated 12 hour intravenous drip of sulfite containing propofol emulsion

In addition to the occurrence of lipid peroxidation and propofol dimerization as discussed previously, sulfite containing propofol emulsion is also known to undergo a discoloration, a yellowing, upon exposure to air[4,5]. The nature of the substance yielding the yellow color as well as the chemical reactions that bring it about are not clear. For that reason, simulated intravenous drip infusion experiments were performed and chemical changes evaluated over time in the emulsions. A total 12 hour drip time was chosen so that emulsion chemistry could be evaluated during hang times 1) that approximate those that occur with the use of the currently marketed 50 and 100 ml vials of 1% propofol emulsion (3-6 hr), and 2) those that will likely occur using larger proposed vials, *ie* 200 ml (6-12+ hr).

In the current study, 50 ml vials of propofol emulsion were dripped using a Flu-Vent vented spike and tubing. Emulsion drip using this system causes air to replace the dripped out emulsion and in such a manner that there is a slow bubbling of air into the emulsion from bottom to top of the liquid. Vials were hung at room temperature under ambient fluorescent lighting. At zero time, 4 ml of emulsion was dripped out and at each hour for

12 hours an additional 4 ml were dripped out and collected. This emptying rate from a 50 ml vial over 12 hr approximates the administration of 2 mg/kg/hr to a 70 kg person from a 200 ml vial of 1% propofol emulsion. The collected emulsion was analyzed for yellowing, MDA formation, and propofol dimerization.

Yellowing of propofol emulsion was determined to occur visibly in a time of 5-7 hours. Initial yellowing was very slight, but noticeable when compared to an unopened vial of sulfite propofol emulsion. The yellowed substance was readily extracted from emulsion with ethyl acetate. The extracted yellow products were chromatographed on a reverse phase HPLC column and the only absorbance detected in the visible range was a band having a maximum absorbance at 424 nm. The formation of this band over the 12 hr simulated intravenous drip continuously increased in the 6 to 12 hr period (Figure 3). Because the violet region (400-430 nm) of the visible electromagnetic spectrum is complementary to yellow, an emulsion having an absorbance band in violet range is expected to appear yellow.

Further investigation showed that the band absorbing at 424 nm chromatographed identically with an authentic compound called propofol dimer quinone (provided by AstraZeneca Pharmaceuticals) which is likewise yellow in solution. A simulated 12 hr intravenous drip of the EDTA propofol emulsion did not result in yellowing of the emulsion. HPLC of the ethyl acetate extracts of EDTA propofol emulsion similarly dripped did not result in any 424 nm bands or any absorbance bands in the visible spectrum during the 12 hr period.

The ethyl acetate extracts from the dripped sulfite containing propofol emulsion were analyzed by electrospray liquid chromatography-mass spectrometry (LC/MS). Figure 4 shows the detection of peaks having ions consistent with propofol dimer (mw = 354) and propofol dimer quinone (mw = 352). Mass spectrums of these peaks confirm the identity of these compounds as propofol dimer and propofol dimer quinone (Figures 5,6,9).

Figure 7 shows the formation of propofol dimer and propofol dimer quinone during the 12 hr drip experiment as evaluated by LC/MS. Low quantities of propofol dimer were present at time zero. Propofol dimer increased over time up to the 12 hrs. Propofol dimer quinone was not detected at time zero and initially appeared at 6 hr. It also showed a continual increase up to 12 hr. The appearance of the propofol dimer quinone correlated with the appearance of emulsion yellowing as determined by the appearance of a 424 nm band by HPLC (Figure 3). These data confirm that sulfite propofol emulsion yellowing upon air exposure is due to propofol dimer quinone.

Analysis of malondialdehyde (MDA) in the sulfite propofol emulsion shows that MDA is detectable at 8 hr in the simulated drip (figure 8). MDA, similar to the propofol dimer and propofol dimer quinone, continued to increase in quantity in the emulsion over time.

The relative intensity of the sulfite free radical signal over time in a simulated 12 hr drip as performed as described above is shown in figure 10. The signal increased from start of

the drip up to 6 hours, followed by a decline. The decline is presumably due to the decline in sulfite concentrations due to its oxidation over time exposed to air.

Discussion

These data demonstrate that under simulated clinical conditions (multi-hour iv drip), sulfite in propofol emulsions will catalyze propofol dimerization and lipid peroxidation in a progressive manner over the 12 hours evaluated. The occurrence of the propofol dimer quinone likely results from the oxidation of the propofol dimer as shown in figure 9. Evidence that propofol dimer and propofol dimer quinone redox cycle can be demonstrated by the addition of a reductant, such as dithionite, to a yellowed sulfite propofol emulsion. This causes a loss of yellow color and increase in propofol dimer formation (data not shown).

The occurrence of these oxidative processes in sulfite propofol emulsion can be attributed to the initial formation of the sulfite radical that is shown here to readily occur in the sulfite containing propofol emulsion. Its occurrence in dripped out emulsion and persistence in a hanging vial shows that it is an infused chemical species during iv drip infusions. The formation of the sulfite radical is a one electron oxidation of sulfite, as opposed to the two electron oxidation of sulfite which forms sulfate[6]. The sulfite radical has the potential to directly react with propofol and unsaturated lipids, or it may form more potent oxidant species in the presence of oxygen such as the sulfite peroxyl radical ($SO_3OO^-$) or sulfate anion radical ($SO_4^-$) [6] to cause emulsion component oxidation.

The significance of MDA measurements is that it is a commonly used marker for lipid peroxidation, and in *in vivo* it is used as a measure of oxidant stress, But it is a minor lipid peroxidation product in lipid systems containing multiple unsaturated fatty acids[8]. MDA arises from the peroxidation of lipids having three or more unsaturations, and as an aldehyde is it a biologically reactive substance[7]. In soybean oil emulsions it predominantly results from linolenic acid (18:3)[8] which constitutes 8% of the emulsion fatty acid lipid[9]. However, other unsaturated fatty acids in propofol emulsions also potentially liberate important lipid cleavage products upon peroxidation. Linoleic acid (18:2) for example, which is present in much higher concentrations (52%) than linolenic acid in propofol emulsions forms the reactive and toxic aldehyde, 4-hydroxy nonenal [7,10]. All the products from sulfite catalyzed lipid peroxidation in propofol emulsions need to be determined.

Summary

Previous work has demonstrated that sulfite under appropriate conditions can act as a prooxidant rather than as an antioxidant[11]. The data presented here show that sulfite in commercial metabisulfite containing propofol emulsion at a concentration of 0.25 mg/ml unexpectedly creates a prooxidant environment when exposed to air.

## Legends for Figures

**Figure 1**. ESR spectrum of commercial metabisulfite containing propofol emulsion. Analysis was performed as described in the text.

**Figure 2**. ESR spectrum of commerical EDTA containing propofol emulsion. Analysis was performed as described in the text.

**Figure 3**. Yellowing of commercial metabisulfite containing propofol emulsion during a simulated 12 hr intravenous drip. One ml of emulsion was extracted with 1 ml ethyl acetate following emulsion cracking with 100µl 10% NaCl. 250 ml of extract was chromatographed on a Waters dual pump HPLC system (Baseline 810 software) equipped with a 484 Millipore absorbance detector. Propofol products were separated on a Hypersil ODS 5 µm column (150 mm x 4.6 mm id) using a solvent of 70% acetonitrile/30% $H_2O$ at a flow rate of 1.5 ml/min. "Absorbance" represents area under the curve (AUC) of the 424 nm band. Brackets indicate approximate time of yellow visualization.

**Figure 4**. LC/MS detection of parent ions of propofol dimer (5.1 min) and propofol dimer quinone (29.8 min) in ethyl acetate extracts of sulfite propofol emulsion. This sample analyzed is a 12 hr sample from the simulated 12 hr infusion. LC/MS analysis was performed on an Agilent Technologies 1100MSD using a C18 Phenosphere NEXT 3 µm column. A mobile phase of 1% formic acid in acetonitrile/water (30/70) was used.

**Figure 5**. Electrospray mass spectrum of propofol dimer from the 12 hr sample (retention time 5.1 min).

**Figure 6**. Electrospray mass spectrum of propofol dimer quinone from the 12 hr sample (retention time 29.8 min).

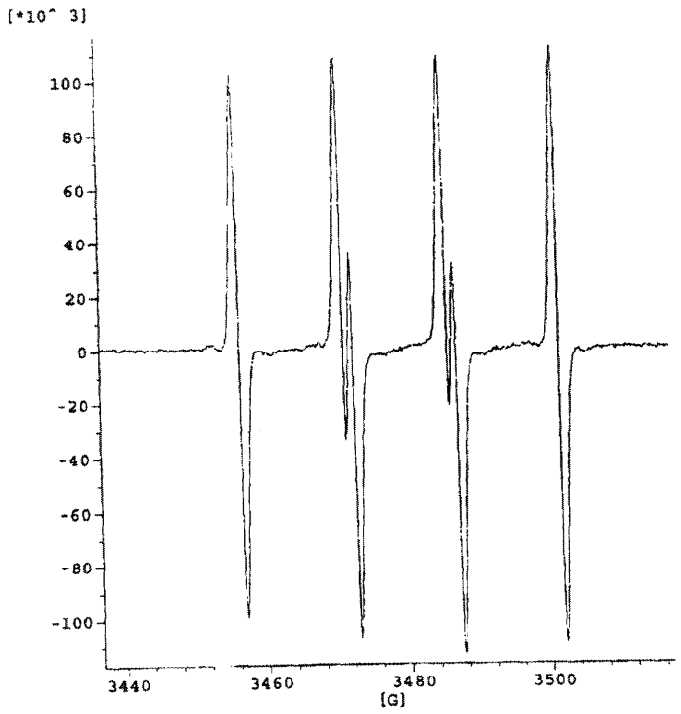
**Figure 7**. Formation of propofol dimer and propofol dimer quinone in sulfite containing propofol during a simulated 12 hr infusion. Analysis was performed by electrospray LC/MS as described in legend to figure 4. Authentic propofol dimer and propofol dimer quinone were used as standards.

**Figure 8**. Malondialdehyde (MDA) formation in sulfite propofol emulsion during a simulated 12 hr intravenous infusion. MDA was analyzed as described in the attached manuscript (Baker et al, Lipid peroxidation in sulfite containing propofol emulsion). Each data set represents a different 50 ml vial of sulfite containing propofol. Each data point represents the mean (±S.E.) of triplicate determinations.

**Figure 9**. Structures of propofol dimer and propofol dimer quinone. Propofol dimer quinone results from the oxidation of propofol dimer.

**Figure 10**. Relative intensity of the ESR signal in sulfite containing propofol emulsion over time in a simulated 12 hr intravenous drip. The simulated 12 hr drip was performed as described in the text.

Figure 1




Parameter List

Operator: SM
Resonator: c:\...\tm9003.cal
Acqu. Date: 11/27/2001
# of Scans: 1

Field
Center Field: 3476.870 G
Sweep Width: 80.000 G
Resolution: 1024 points

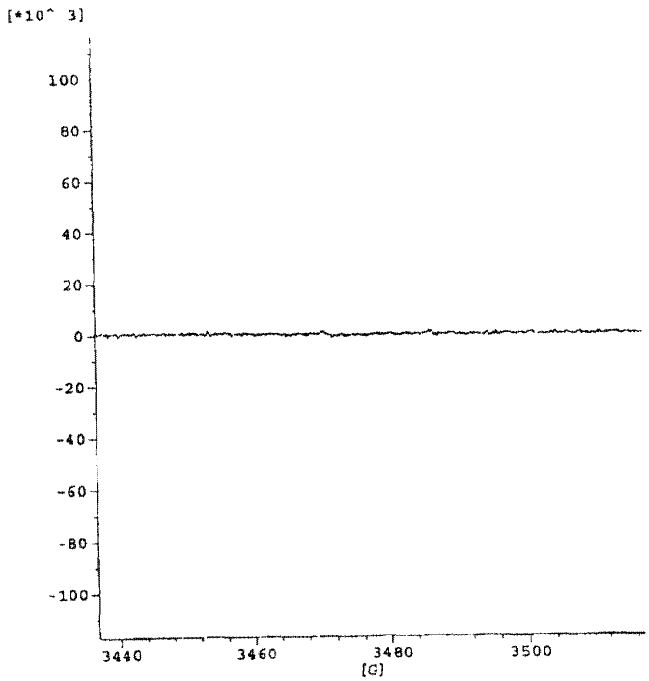
Microwave
Frequency: 9.766 GHz
Power: 40.140 mW

Receiver
Receiver Gain: 1.00e+005
Phase: 0.00 deg
Harmonic: 1
Mod. Frequency: 100.00 kHz
Mod. Amplitude: 1.00 G

Signal Channel
Conversion: 327.680 ms
Time Constant: 327.680 ms

Sweep time 355.445 s

Figure 2




Parameter List

| | |
|---|---|
| Operator: | SM |
| Resonator: | c:\...\tm9003.cal |
| Acqu. Date: | 11/27/2001 |
| # of Scans: | 1 |

Field

| | |
|---|---|
| Center Field: | 3476.870 G |
| Sweep Width: | 80.000 G |
| Resolution: | 1024 points |

Microwave

| | |
|---|---|
| Frequency: | 9.785 GHz |
| Power: | 40.240 mW |

Receiver

| | |
|---|---|
| Receiver Gain: | 1.00e+005 |
| Phase: | 0.00 deg |
| Harmonic: | 1 |
| Mod. Frequency: | 100.00 kHz |
| Mod. Amplitude: | 1.00 G |

Signal Channel

| | |
|---|---|
| Conversion: | 327.680 ms |
| Time Constant: | 327.680 ms |
| Sweep Time: | 335.544 s |

Figure 3

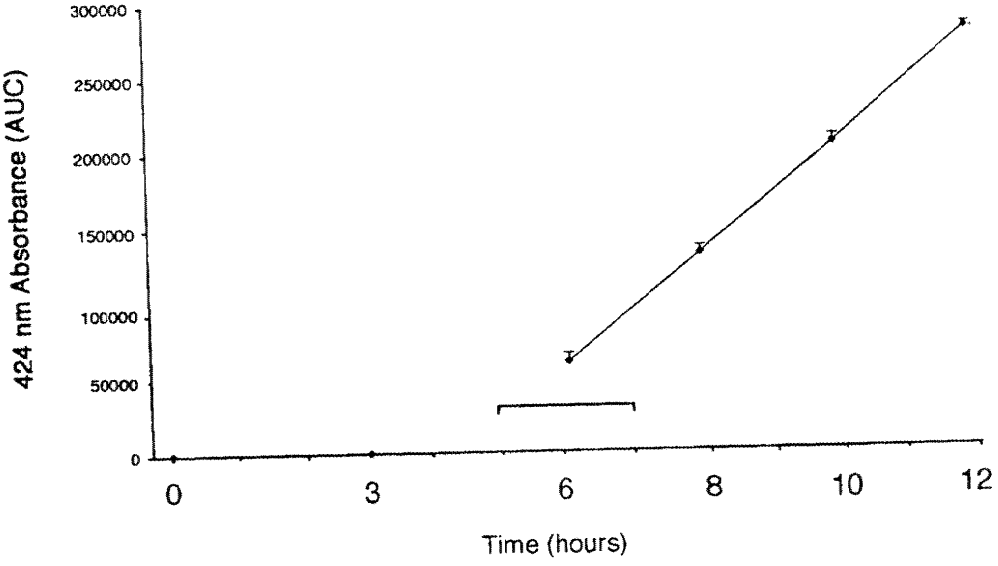
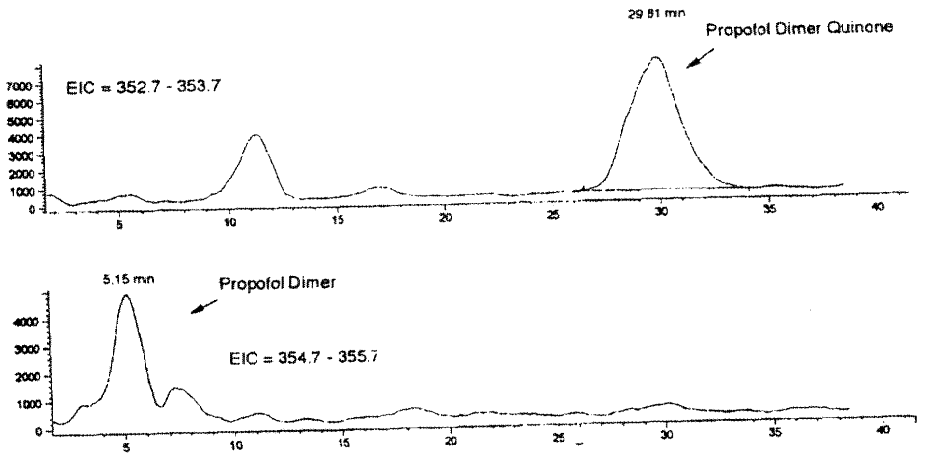

424 nm Absorbance (AUC)
300000
250000
200000
150000
100000
50000
0
0
3
6
8
10
12
Time (hours)

Figure 4




retention time (min)

Figure 5

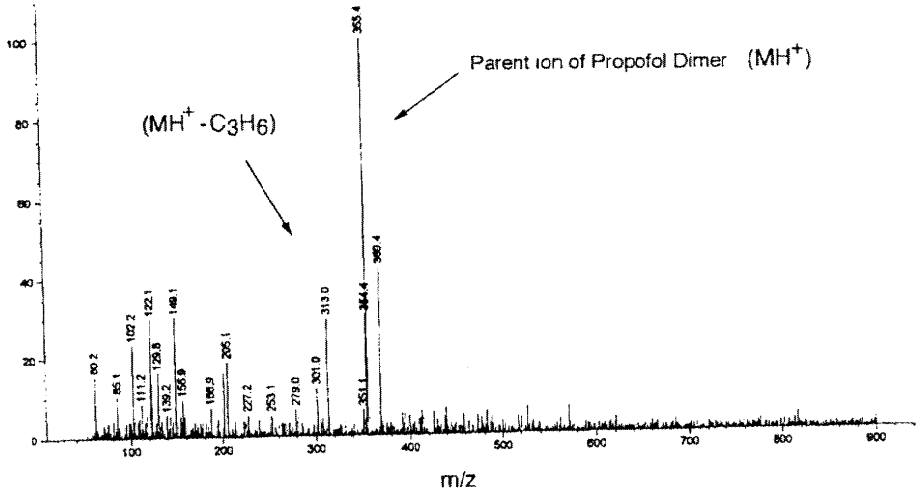


Figure 6

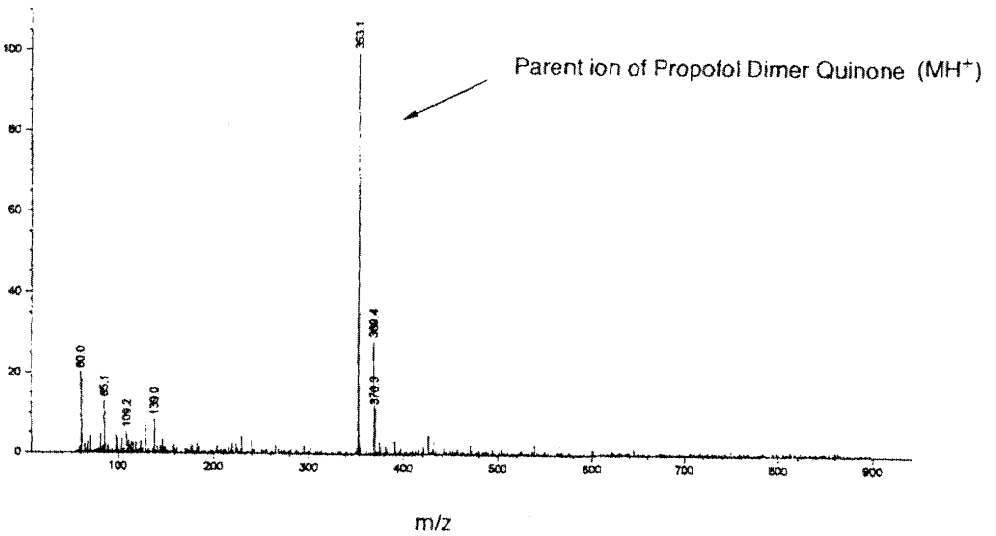

Parent ion of Propofol Dimer Quinone (MH+)
353.1
369.4
80.0
85.1
109.2
130.0
100
80
60
40
20
0
100
200
300
400
500
600
700
800
900
m/z

Figure 7

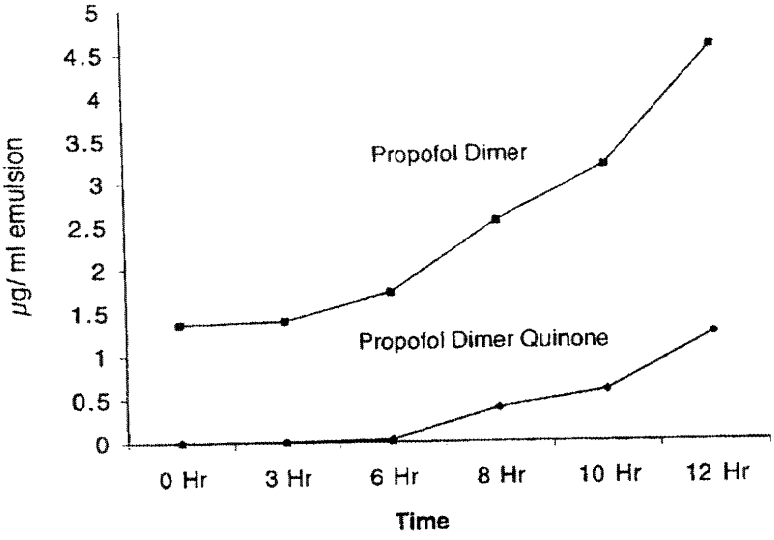

5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
µg/ ml emulsion
Propofol Dimer
Propofol Dimer Quinone
0 Hr
3 Hr
6 Hr
8 Hr
10 Hr
12 Hr
Time

Figure 8

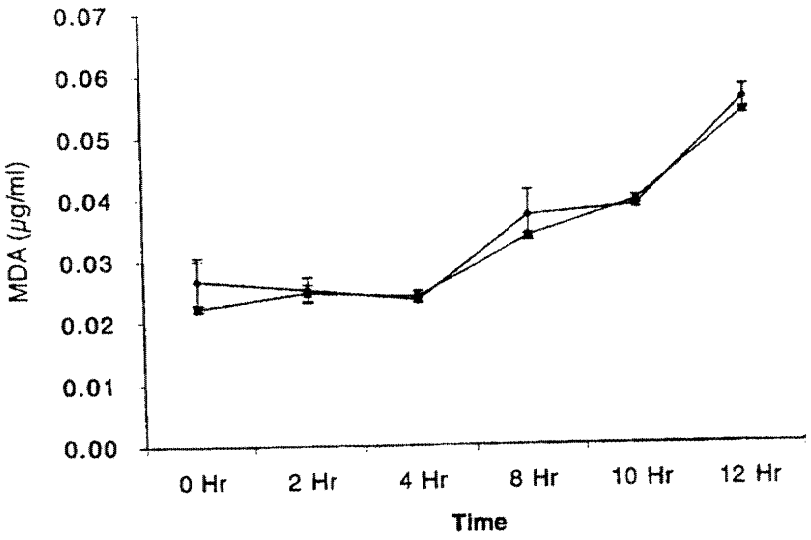
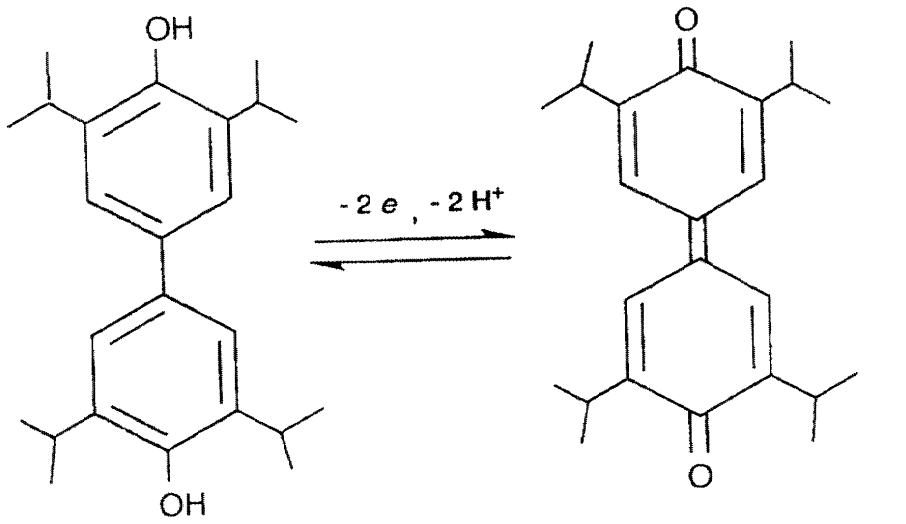

0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
MDA (µg/ml)
0 Hr
2 Hr
4 Hr
8 Hr
10 Hr
12 Hr
Time

Figure 9



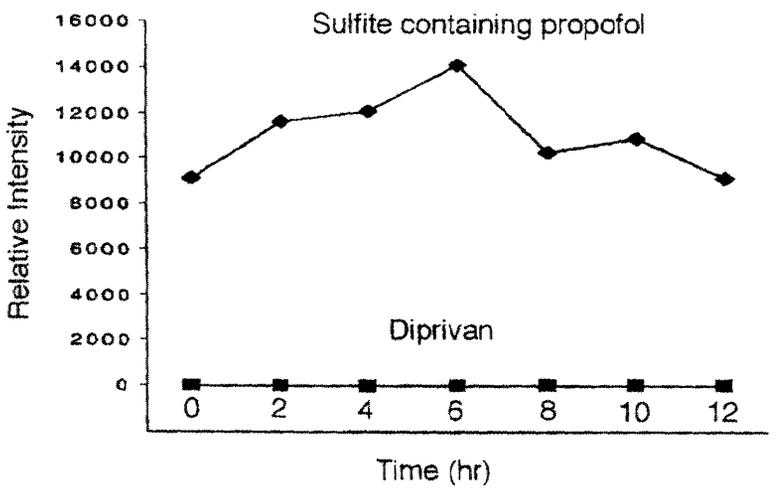

Propofol Dimer

MW = 354

Propofol Dimer Quinone

MW = 352

Figure 10


Sulfite containing propofol
Diprivan
Relative Intensity
16000
14000
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12
Time (hr)

## References


1. Baker MT. Comparison of emulsion chemistry between sulfite-containing propofol and propofol with EDTA. *Am J Anesth* (Suppl) 2000; 27:19-21.

2. Sun X, Shi X, Dalal NS. Xanthine oxidase/hydrogen peroxide generates sulfur trioxide anion radical ($SO_3^{\cdot}$) from sulfite ($SO_3^{\cdot 2}$). *Fed Eur Biochem Soc* 1992; 303, 213-6.

3. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG. The antioxidant potential of propofol (2,6-diisopropylphenol). *Brit J Anaes* 1992; 68:613-18.

4. Baker MT. Yellowing of metabisulfite-containing propofol emulsion. *Am J Health-Syst Pharm.* 2001; 58: 1042-4.

5. Mirejovsky D. Yellowing of metabisulfite-containing propofol emulsion (reply). *Am J Health-Syst Pharm.* 2001; 58:1046-7.

6. Mottley C and Mason RP. Sulfate anion free-radical formation by the peroxidation of (bi)sulfite and its reaction with hydroxyl radical scavengers. *Arch Biochem Biophys* 1988; 267:681-9.

7. Koh Yh, Yoon SJ, Park JW. Lipid peroxidation product-mediated DNA damage and mutagencity. *J Biochem Mol Biol.* 1997;30:188-193.

8. Mead JF, Alfin-Slater RB, Howton DR, Popjak G. Peroxidation of fatty acids (Chap 6), In lipids, Chemistry, Biochemistry and Nutrition. Plenum Press, New York, NY, 1986.

9. Wu GH, Jarstrand C, Nordenstrom J. Phagocyte-induced lipid peroxidation of different intravenous fat emulsions and counteractive effect of vitamin E. *Nutrition* 1999; 15:359-64.

10. Tamura H Shibamoto T. Gas-chromatographic analysis of malonaldehyde and 4-hydroxy-2-(E)-nonenal produced from arachadonic-acid and linoleic-acid in a lipid peroxidation model system. *Lipids* 1991;26:170-73.

11. Lavoie J-C, Lachance C and Chessex P. Antiperoxide activity of sodium metabisulfite. A double-edged sword. *Biochem Pharmacol* 1994; 47:871-76.

# COLLEGE OF MEDICINE CURRICULUM VITAE

Max T. Baker

8-17-2001

## • EDUCATIONAL AND PROFESSIONAL HISTORY

1975 B.S. in Biology, Georgia College, Milledgeville, GA.

1978 M.S. in Pharmacology, University of Georgia, Athens, GA. Thesis Title: Effects of Dietary thiamin on the metabolism of benzo(a)pyrene in rats hepatic microsomes. Thesis Advisor: Adelbert E. Wade, School of Pharmacy, University of Georgia, Athens, GA

1980 Ph.D. in Pharmacology, University of Georgia, Athens, GA. Thesis Title: The effects of dietary lipids on the metabolism and activation of benzo(a)pyrene by mouse hepatocytes nuclei and microsomes. Thesis Advisor: Adelbert E. Wade, Ph.D. School of Pharmacy, University of Georgia, Athens, GA

1980-1983 Research Fellow, Department of Anesthesiology Mayo Graduate School of Medicine, Rochester, MN.

1983-1984 Research Associate, Department of Anesthesiology, Mayo Graduate School of Medicine, Rochester, MN. (Academic instruction in Pharmacology)

1984-1989 Associate Research Scientist, Department of Anesthesia, University of Iowa College of Medicine, Iowa City, IA (Academic instruction in anesthesia/toxicology)

1989-1994 Assistant Professor, Department of Anesthesia, University of Iowa College of Medicine, Iowa City, IA

1994-present Associate Professor (with tenure), Department of Anesthesia, University of Iowa, College of Medicine, Iowa City, IA.

- **SCHOLARSHIP**

A Publications

1. **Baker MT**, Karr SW and Wade AE: The effects of dietary corn oil on the metabolism and activation of benzo(a)pyrene by the benzo(a)pyrene metabolizing enzymes of the mouse. *Carcinogenesis* 4:9-15, 1983.

2. **Baker MT**, Nelson RM and Van Dyke RA: The release of inorganic fluoride from halothane and halothane metabolites by cytochrome P-450, hemin, and hemoglobin. *Drug Metab. Dispos.* 11:308-311, 1983.

3. Wade AE, Evans JS, Holmes DT and **Baker MT**: The influence of dietary thiamin on phenobarbital induction of rat hepatic enzymes responsible for metabolizing drugs and carcinogens. *Drug Nutrient Interact.* 2:117-130, 1983.

4. **Baker MT** and Van Dyke RA: Metabolism-dependent binding of the chlorinated insecticides, DDT and DDD, to cellular macromolecules. *Biochem. Pharmacol.* 33:255-260, 1984.

5. **Baker MT** and Van Dyke RA: Reductive halothane metabolite formation and binding in rat hepatic microsomes. *Chem-Biol. Interact.* 49:121-132, 1984.

6. Wade AE, Bunce OR, Harley W, **Baker MT** and Holmes D: Effect of thiamin deprivation on the in vitro metabolic activation of benzo(a)pyrene. *Pharmacology* 29:142-148, 1984.

7. **Baker MT**, Nelson RM and Van Dyke RA: The formation of chlorobenzene and benzene by the reductive metabolism of lindane by rat liver microsomes. *Arch. Biochem. Biophys.* 236:506-514, 1985.

8. **Baker MT**, Bates JN, and Van Dyke RA: Stabilization of the reduced halocarbon-cytochrome P-450 complex of halothane by n-alkanes. *Biochem. Pharmacol.* 36:1029-1034, 1987.

9. **Baker MT**, Bates JN and Leff SV: Comparative defluorination and cytochrome P-450 loss by the microsomal metabolism of fluoro- and fluorochloroethenes. *Drug Metab. Dispos.* 15:499-503, 1987.

10. **Baker MT**, Bates JN, and Leff SV: Stimulatory effects of halothane and isoflurane on fluoride release and cytochrome P-450 loss caused by metabolism of 2-chloro-1,1-difluoroethene, a halothane metabolite. *Anesth. Analg.* 66:1141-1147, 1987.

11. **Baker MT** and Bates JN: Metabolic activation of the halothane metabolite, [$^{14}$C]-2-chloro-1,1-difluoroethene, in hepatic microsomes. *Drug Metab. Dispos.* 16:169-172, 1988.

12. Van Dyke RA, **Baker MT**, Jansson I and Schenkman J: Reductive metabolism of halothane by purified cytochrome P-450. *Biochem. Pharmacol.* 37:2357-2361, 1988.

13. **Baker MT**, Vasquez MT, Bates JN and Chiang CK: Metabolism of 2-Chloro-1,1-difluoroethene to Glyoxylic and Glycolic Acid in Rat Hepatic Microsomes. *Drug Metab. Dispos.* 18:753-758, 1990.

14. Hansen TD, Warner DS, Todd MM, **Baker MT** and Jensen NF: The influence of inhalational anesthetics on in vivo and in vitro benzodiazepine receptor binding in the rat. *Anesthesiology.* 74:97-104, 1991.

15. **Baker MT**, Vasquez MT and Chiang CK: Evidence for the Stability and Cytochrome P-450 Specificity of the Phenobarbital Induced Halothane-Cytochrome P-450 Complex Formed in Rat Hepatic Microsomes. *Biochem. Pharmacol.* 41:1691-1699, 1991.

16. Ridenour TR, Warner DS, Todd MM, **Baker MT**: Effects of Ketamine on Outcome from Temporary Middle Cerebral Artery Occlusion in the Spontaneously Hypertensive Rat. *Brain Research.* 565:116-122, 1991.

17. Bates JN, **Baker MT**, Guerra R, Harrison DG: Nitric oxide generation from nitroprusside by vascular tissue: evidence that reduction of the nitroprusside anion and cyanide loss are required. *Biochem. Pharmacol.* 42:S157-S165, 1991.

18. **Baker, M.T.** and R.A.Van Dyke: Biochemical and toxicological aspects of the volatile anesthetics. In Clinical Anesthesia, 2nd Edition, 1992, P.G.Barash, B.F.Cullen and R.K.Stoelting eds. J.P.Lippincott Co. Chapter 18, pp 467-480.

19. **Baker, M.T.** and W.C.Ronnenberg,Jr.: Acute stimulation of trifluoroethene defluorination and cytochrome P450 inactivation in the rat by exposure to a sub-anesthetic concentration of isoflurane. *Tox. Appl. Pharmacol.* 114:25-30, 1992.

20. Speigel, D.A., D.S.Warner, F. Dexter, **M.T.Baker**, and M.M.Todd: Central nervous system toxicity of local anesthetic mixtures in the rat. *Anesth. Analg.* 75:922-928, 1992.

21. **Baker, M.T.**, M.V.Chadam, and W.C.Ronnenberg, Jr: Inhibitory effects of propofol on cytochrome P450 activities in the rat. *Anesth. Analg.* 76:817-821, 1993.

22. Wang, Y. and **M. T. Baker**: NADPH and oxygen consumption in isoflurane facilitated 2-chloro-1,1-difluoroethene metabolism in rabbit liver microsomes. *Drug Metab and Dispos.* 21: 299-304, 1993.

23. **Baker, M.T.** and W. C. Ronnenberg, Jr.: Contrasting effects of 1,1,1-trichloroethane on $^{14}$C-vinyl chloride metabolism and activation in hepatic microsomes from phenobarbital and isoniazid treated rats. *Tox. Appl. Pharmacol* 119:17-22, 1993.

24. Wang, Y., M.J.Olson and **M.T.Baker**: Interaction of fluoroethane CFC substitutes with microsomal cytochrome P450: Stimulation of P450 activity and chlorodifluoroethene metabolism. *Biochem. Pharmacol.* 46:87-94, 1993.

25. **Baker, M.T.**, C-K Chiang and J.H.Tinker: Synthesis of fluoro-dideutero-methyl 1,1,1,3,3,3-hexafluoro-2-propyl ether (deuterated sevoflurane). *J.Label. Compound Radiopharma.* 33:801-807, 1993.

26. **Baker, M.T.**, W. C. Ronnenberg, Jr, C-K.Chiang, J. A. Ruzicka and J.H.Tinker. The effect of deuterium substitution on the metabolism of sevoflurane by the rat. *Drug Metab. Dispos.* 21:1170-1171, 1993.

27. Ruzicka, J.A., W. Qiu, **M.T. Baker**, and D.J. Burton: Synthesis of 2-$^{14}$C-1,1-difluoroethene from $^{14}$C-formaldehyde. *J.Label. Compound Radiopharma.* 34:59-65, 1994.

28. Ruzicka, J.A. Higaldo, J., Tinker, J.H. and **M.T.Baker**: Inhibition of volatile sevoflurane degradation product formation in an anesthesia circuit by a reduction in soda lime temperature. *Anesthesiology* 81:238-244, 1994.

29. Ruzicka, J.A. and **M.T. Baker**: Fluoro-dideutero-methyl 1,1,1,3,3,3-hexafluoroisopropyl ether (D2-sevoflurane) reactions on soda lime: Deuterium content of deuterated sevoflurane and its volatile degradation products. *J.Fluorine Chem.* 71:55-58, 1995.

30. **Baker, M.T.**, M.J.Olson, Y. Wang, W.C. Ronnenberg, J.T.Johnson and A. N. Brady: Isoflurane - chlorodifluoroethene (CDE) interaction in human liver microsomes: Role for cytochrome P450 2B6 in potentiation of haloethene metabolism. *Drug Metab Dispos* 23:60-64, 1995.

31. Ronnenberg, W. C. Jr., Y. Wang and **M. T. Baker**: Isoflurane and cytochrome $b_5$ stimulation of 2-chloro-1,1-difluoroethene (CDE) in reconstituted rat cytochrome P450 2B1 and CYP2C6. *Biochem Pharmacol* 50:521-528, 1995.

32. Ruzicka, J.A., J.B. Hrdy and **M.T. Baker**: Synthesis and pharmacological properties of 1,2-bis(fluoromethoxy)-1,1,3,3,3-pentafluoropropane. *J Fluorine Chem* 75:191-196, 1995.

33. Ruzicka, J.A., S. D. Pederson, and **M.T.Baker**: Synthesis and toxicity of 1,2-bis(trifluoromethoxy)-1,1,3,3,3-pentafluoropropane. *J Fluorine Chem* 76:213-217, 1996.

34. Kim, Y.J., C. McFarlane, D.S.Warner, **M.T.Baker**, W.W.Choi, and F. Dexter: Chronic Hypermagnesemia does not alter the threshold for lidocaine induced seizures in the rat (*Anesth Analg*, 1996).

35. **Baker, M.T.**, W.C. Ronnenberg, Jr. and J.A. Ruzicka. Deuterium Labeling of methyl 1,1,1,3,3,3-hexafluoroisopropyl ether. *J. Label Compound Radiopharma,* 39:387-393, 1997).

*36.* **Baker, M.T.**, J.A.Ruzicka and J.H.Tinker. One step synthesis of 1,1,1,3,3,3-hexafluorobutane from succinonitrile (*J. Fluorine Chem.* 94: 123-126, 1999).

*37.* **Baker, MT.** Comparision of emulsion chemistry between sulfite containing propofol and propofol with EDTA. Am. J. Anesthesiology. 27 (6S): 19-21, 2000.

38. **Baker, MT**. Role of lipids in propofol dimerization in propofol emulsions containing metabisulfite (in preparation).

39. Ruzicka, J.A., J.H.Tinker and **M.T.Baker.** A novel process for the synthesis of 1,1,1,3,3,3-hexafluoropropane (submitted to *Synthesis*).

40. **Baker, MT**. Oxidation of propofol in propofol emulsions containing metabisulfite (in preparation).

Chapters:

1 **Baker, M.T.** and R.A.Van Dyke: Hepatotoxicity of Halogenated Anesthetics (Chapter). In Anesthetic Toxicity. Editors: S.Rice and K. Fish. Raven Press, 1994.

2 **Baker, M.T.** and R.A.Van Dyke: Biochemical and toxicological aspects of the volatile anesthetics. In Clinical Anesthesia, 2nd Edition, 1992, P.G.Barash, B.F.Cullen and R.K.Stoelting eds. J.P.Lippincott Co. Chapter 18, pp 467-480.

3 **Baker, M.T.:** Toxicity of the Volatile Anesthetic Agents (Chapter 17). In Comprehensive Toxicology, Vol 9. Editors: I.G.Sipes, C.A. McQueen, A.J.Gandolfi. Elsevier Science, 1997, pp 295-308.

4 **Baker, M.T.** and D.F.Halpern: The Development and synthesis of the Fluorinated Anesthetics. (review article, in preparation).

5 **Baker M.T.** and J. A. Ruzicka. Reactions of bromine trifluoride. A review. (to be submitted to J. Fluorine Chem).

**B. Abstracts** (partial listing):

1. **Baker MT** and Wade AE: The effects of dietary thiamin on the metabolism of benzo(a)pyrene in rat hepatic microsomes. *Fed.Proc.* 37:271,1978.

2. **Baker MT** and Wade AE: The effects of dietary lipids on the metabolism and activation of benzo(a)pyrene by mouse hepatocyte nuclei and microsomes. *Fed.Proc.* 40:949,1980.

3. **Baker MT**, Carlson GL and Van Dyke RA: Metabolism-dependent covalent binding of DDT and lindane to cellular macromolecules. *The Pharmacologist* 23:173,1981.

4. **Baker MT** and Van Dyke RA: Metabolism-dependent binding of DDT and DDD to microsomal components. *Fed.Proc.* 41:1054,1982.

5. **Baker MT** and Van Dyke RA: Reduction of halothane by heme and hemeprotein. *Fed.Proc.*42:1143,1983.

6. **Baker MT** and Van Dyke RA: Benzene and chlorobenzene are lindane metabolites. *Toxicol. Lett.* 18:65,1983.

7. Van Dyke RA, **Baker M**, Jansson I and Schenkman J: Studies of halothane-cytochrome P-450 complex and metabolite formation in a purified P-450 system. *Fed.Proc.* 44:1468, 1985.

8. **Baker MT** and Van Dyke RA: Properties of the reduced halothane-cytochrome P-450 complex. *Fed.Proc.* 44:1468,1985.

9. **Baker MT**, Bates JN and Leff SV: Metabolism-dependent inhibition of cytochrome P-450 by the halothane metabolite, chlorodifluoroethylene, and chlorotrifluoroethylene. *Fed. Proc.* 45:576,1986.

10. Bates JN and **Baker MT**: The effects of anesthetics on fluoride release and cytochrome P-450 loss by the metabolism of chlorodifluoroethene. *Anesthesiology* 65:A234,1986.

11. Bates JN and **Baker MT**: The decomposition of sodium nitroprusside by blood components. *Anesthesiology*, 67:A307, 1987.

12. **Baker MT**, Suter DM and Bates JN: Formation of hexafluoroisopropanol from the microsomal metabolism of sevoflurane. *Anesthesiology*, 67:A293, 1987.

13. Bates JN, Myers PR, Guerra R, **Baker MT**, Harrison D: Synthesis and detection of S-Nitroso-L-Cysteine. *Fed. Proc.* 3:A1144, 1989.

14. Hansen TD, Warner DS, Todd MM, **Baker MT**, Jensen NF: The influence of inhalational anesthetics on in vivo and in vitro benzodiazepine receptor binding in the rat. *Anesthesiology* 71:A1204, 1989.

15. **Baker MT**, Vasquez MT, Bates JN, Chiang CK: The metabolism of chlorodifluoroethene to glycolic acid. *Anesthesiology* 71:A241, 1989.

16. **Baker MT**, Vasquez MT, Chiang CK: Effects of Halothane Reduction of the Regiospecific Metabolism of R- and S-Warfarin in Hepatic Microsomes. *Anesthesiology*, In press.

17. **Baker, M.T** and William C. Ronnenberg,Jr.: Acute Stimulation of trifluoroethene defluorination and cytochrome P450 inactivation in the rat by exposure to a sub-anesthetic concentration of isoflurane. *Pharmacologist.* 33, 1991

18. Wang Y, **Baker MT**, and Olson MJ: Stimulation of microsomal P450 activity and chlorodifluoroethene (CDE) metabolism by 1,1,1-trifluoroethane freon substitutes. *Toxicologist.* 13:345, 1993.

19. **Baker MT**, Wang Y, and Olson MJ: Isoflurane potentiation of 2-chloro-difluoroethene (CDE) metabolism in human liver microsomes. *Toxicologist.* 13:431, 1993.

20. Wang Y, and **Baker MT**: Isoflurane (ISO) and halothane (HAL) potentiation of 2-chloro-1,1-difluoroethene (CDE) metabolism and hepatotoxicity in phenobarbital-treated rats. *Toxicologist 1993.*

21. Naguib M, Hammond DL, **Baker M**, Cutkomp J, Queral L. Induction of general anesthesia by melatonin: Comparative dose-effect studies with thiopental and propofol. Anesthesiology (A-1373, 2001).

22. **Baker MT.** Thiobarbituric acid reactive substances (malondialdehyde) in metabisulfite containing propofol emulsion. Anesthesiology (A-503, 2001)

C. Patents

1. **Baker, M.T.** and J.H.Tinker. Deuterated sevoflurane as an inhalational anesthetic. **US Patent #5,391,579***, issued February 21, 1995 to the University of Iowa. (UIRF #93-26 - Serial No. 08/010,264, filed Jan. 28, 1993. Claims allowed July 18, 1994).

2. Tinker, J.H. and **M.T.Baker**. A method for the elimination of the degradation of sevoflurane and deuterated sevoflurane on soda lime. **US Patent #5,492,111**, issued February 20, 1996. (filed Jan. 28, 1993, Claims allowed June 8, 1995).

3. **Baker, M.T.** and J.H.Tinker. Deuterated sevoflurane as an inhalational anesthetic. **US Patent #5,789,450**, August 4, 1998

4. **Baker, M.T.**, J.H. Tinker and J.A. Ruzicka. Process for the synthesis of hexafluoroisopropyl ethers. (filed January 1997, claims allowed July 15, 1997). **US Patent #5,705,710***, Jan. 6, 1998

5. Baker, M.T. and J.A. Ruzicka, Process for the synthesis of Hexafluoropropanes. **US Patent # 5,789,630,** Aug. 4, 1998.

6. **Baker, M.T.** and J.H.Tinker. A method for the synthesis of deuterated sevoflurane. (application filed - continuation of UIRF #93-26 - Serial No. 08/010,264, filed Jan. 28, 1993).

7. **Baker, M.T**. Propofol emulsions containing ascorbate, assignee Astrazeneca Pharmaceuticals, Inc (applied for Sept 2001).

* Foreign patent applications filed.

Active Document Disclosures:

1. **Baker, M.T.** Fluorinated alkyl phenol compounds and their use as medicinal agents (7-5-01, DD# 496474)

2. **Baker, M.T**. A method for the administration of propofol to mammals by inhalation (5-29-01, DD# 494391).

3. **Baker, MT**. A method for the andministration of propofol to mammals in subanesthetic doses (6-1-01, DD# 494588).

D. Letters

Tinker, J.H. and **Baker, M.T**. Sevoflurane, Fluoride Ion and Renal Toxicity. (Letter to the editor) *Anesthesiology* 83:232-233, 1995.

**Baker, MT**. Comparative propofol emulsion stabilities. *AANA Journal* 68:287, 2000.

**Baker MT**. Yellowing of the propofol emulsion containing sulfite *Am. J. Health-System Pharmacists* , 58: 1042-1044, 2001.

## B. Areas of Research Interest and Current Projects

Basic Interest: Drug Metabolism. Anesthetic Metabolism; Mechanisms of Anesthetic Liver Toxicity; Influence of Anesthetics on Drug Metabolizing Reactions. Anesthetic synthesis. Anesthetic and fluorocarbon drug development.

Anesthetic/Fluorocarbon cytochrome P450 Interactions

- Interaction of fluorinated anesthetics, fluorinated CFC substitutes, and fluorinated ethenes (industrial monomers) with the cytochrome P450 drug metabolizing enzymes of rats, rabbits and humans.

Emphasis is on the ability of saturated fluoroalkanes and ethers to stimulate uncoupled P450 activity and cause a metabolic switching mechanism whereby saturated fluorocarbons stimulate gaseous haloethene metabolism. Emphasis is also on identification of the specific P450 isozymes in rats and humans that exhibit fluoroalkane potentiated haloethene metabolism.

- Hepatotoxicity and metabolism of fluorinated and deuterated compounds in the rat.

Emphasis is on the interactive effects of fluorocarbon ethers on liver injury by haloethenes, and correlation of interactive toxicity with fluoroalkane stimulated haloethene metabolism. Metabolic pathways and activation of 1,1-difluoroethene and trifluoroethene, and their potent P450 inactivating properties are also being investigated.

- Synthesis and development of volatile anesthetics and other fluorinated compounds.

Emphasis is on the development and testing of deuterated sevoflurane as a volatile anesthetic, and the development of chemical methods for the synthesis of the hexafluoropropyl group on several compounds including sevoflurane. In addition, we have successfully synthetized a novel fluorinated diethyl compound which exhibits anesthetic effects. We have developed a new method for the synthesis of sevoflurane related compounds including hexafluoropropane and hexafluorobutane.

- The role of deuterium isotope effect on the liver toxicity on therapeutic CNS compound valproic acid.

**C. Current Projects:**

- Propofol emulsion stability in the presence of bisulfite additives
Propofol chemical degradation in formulations containing the antioxidant metabisulfite.

- Synthesis of melatonin derivatives (eg bromomelatonin) and the anesthetic properties of melatonin and its analogues (with M. Naguib).

D Grants and Contracts:

NIH (R29-GM41121-01A2) (PI Max T. Baker, 70%) Fluorinated ethene metabolism and hepatotoxicity in the rat (7/01/90 - 7/01/95).

NIH (1 T32 GM08442-01) (Program Faculty, 5%) Multidisciplinary Anesthesia Research Training Program

Contract for plasma analysis for protocol GHBA-525 PI Max T, Baker (A phase III, multicenter, randomized, open-label study to compare the safety and tolerability of sevoflurane administered with nitrous oxide and oxygen in ASA class I, II, and III patients). Maruishi Pharmaceuticals Co., LTD. Study conducted by G.H.Besselaar Associates. Princeton, NJ. 11-91 - 11-92. (Study expanded 1/6/92)

Quality control fluoride analyses for Abbott Laboratories. PI Max T. Baker (8-93). A multicenter phase III study of sevoflurane metabolism in surgical patients.

Processes for the synthesis of deuterated sevoflurane. PI Max T. Baker From Abbott Labs (via Tinker and Baker Labs) PI Max T. Baker (Jan 1, 1997-Dec. 31,1997).

Formulation difference tests of Diprivan and propofol with metabisulfite. PI, Max T. Baker. From Astra/Zeneca Pharmaceuticals. (Dec 1999 to Mar 2000).

Chemical Grant of Deuterated Valproic acid, September 1999, from Cambridge Isotope Chemicals .

Formulation difference tests of Diprivan and propofol with metabisulfite (renewal), PI, Max T. Baker, From AstraZeneca Pharmaceuticals August 1, 2000-August 1, 2001.

A Clinical Investigation of Unknown Exhaled Substances in Patients Administered Propofol Emulsion Containing Metabisulfite, PIs Max T. Baker and Deborah Dehring. From AstraZeneca Pharmaceuticals January 1, 2001-January 1, 2002.

E Invited lectures, conference presentations.

Max T. Baker. Relative stability of Diprivan and propofol with metabisulfite in the shake test. Presentation to the Diprivan advisory board, Dec. 2, 1999, Orlando, FL.

Max T. Baker. Chemistry of propofol emulsions containing metabisulfite, Diprivan Advisory Board. July 12, 2000, Washington DC.

Max T. Baker. Chemical processes in metabisulfite containing propofol emulsion. FDA seminar on emulsion chemistry. August 24, 2000, Rockville, MD.

Max T. Baker. Role of sulfite in propofol emulsion chemistry, AstraZeneca propofol Investigators meeting. San Diego, March 10, 2001.

- SERVICE

Education Committee, University of Iowa Department of Anesthesia (1992-1996).

Steering Committee, University of Iowa College of Medicine Nurse Anesthetist Training Program (April 1994 to 1997)

Ph.D. Thesis Committee (April 1996): Scot Pedersen, Department of Chemistry

Chemical Safety Officer, Department of Anesthesia University of Iowa (1991-2000).

College of Medicine Research Committee, University of Iowa College of Medicine (1994-1999).

Continuing Education Committee, University of Iowa College of Medicine (1999- present).

Nurse Anesthetist Training Program Admissions Committee, University of Iowa, Departmental of Anesthesia and department of Nursing (2000-present).

Peer reviewer for the Journals: Anesthesiology, Anesthesia & Analgesia, Biochemical Pharmacology, Drug Metabolism and Disposition, Toxicology and Applied Pharmacology, Toxicology.